Pharmacology of botulinum toxin

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Background: Botulinum toxin has a well-defined role among dermatologists for the treatment of facial wrinkling, brow position, and palmar and axillary hyperhidrosis.

Objective: The purpose of this study is to educate dermatologists on the pharmacology of botulinum toxin.

Methods: A retrospective review of the literature on botulinum toxin from 1962 to the present was conducted. We examined the clinical applications of botulinum toxin, cholinergic neuromuscular transmission, the toxin's structure and molecular actions, drug and disease interactions at the neuromuscular junction, toxin assays, determinants of clinical response, and adverse side effects.

Results: Botulinum toxin blocks the release of acetylcholine from the presynaptic terminal of the neuromuscular junction. Several drugs and diseases interfere with the neuromuscular junction and the effects of botulinum toxin. The mouse bioassay, the most sensitive and specific measurement of toxin activity, is the gold standard for botulinum toxin detection and standardization. The major determinants of clinical response to treatment are the toxin preparation, individual patient's anatomy, dose and response relationships, length of toxin storage after reconstitution, and immunogenicity. To minimize potential antibody resistance, one should use the smallest effective dose, utilize treatment intervals of more than 3 months, and avoid booster injections. Uncommon adverse effects include ptosis, ectropion, diplopia, bruising, eyelid drooping, hematoma formation, and temporary headaches.

Conclusion: Botulinum toxin is a safe and effective treatment. Knowledge of the pharmacologic basis of therapy will be useful for standardizing techniques and achieving consistent therapeutic results in the future. (J Am Acad Dermatol 2000;43:249-59.)

BACKGROUND

Neurotoxins produced by the gram-positive, anaerobic *Clostridium botulinum* are the most potent toxins known to mankind and are the causative agents of botulism. Botulinum toxin (BTX) acts by blocking the release of acetylcholine from the presynaptic terminal of the neuromuscular junction. Seven distinct antigenic botulinum toxins (BTX-A, B, C, D, E, F, and G) produced by different strains of *Clostridium botulinum* have been described.[1] The human nervous system is susceptible to 5 toxin serotypes (BTX-A, B, E, F, G) and unaffected by 2 (BTX-C, D).[2]

There are 3 forms of human botulism: food-borne, infantile, and wound.[3] Classic or food-borne botulism results from the ingestion of food containing preformed neurotoxin types A, B, or E.[4] It is characterized by a symmetric, descending flaccid paralysis of motor and autonomic nerves, usually beginning with the cranial nerves. Blurred vision, dysphagia, and dysarthria are common initial symptoms and in the severest cases can lead to respiratory arrest and death.[5] Infant botulism is caused by ingestion of spores and production of toxin in the infant's intestine. Wound botulism arises as a consequence of toxin produced in wounds contaminated with the organism.[6]

The potential medical applications of BTX-A were first recognized by Scott[7] in the early 1980s when he used local injection of minute doses to selectively inactivate muscle spasticity in strabismus. BTX-A was found to be a safe and effective therapy without significant local or systemic side effects. This success and a series of clinical studies[8-11] led to Food and Drug Administration (FDA) approval in 1989 for ophthalmologic and neurologic use in strabismus, blepharospasm, and hemifacial spasm. BTX-A has since been used in other conditions related to excessive

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Table I. Clinical use of Botox

Dermatology	Ophthalmology	Neurology
Glabellar frown lines	Strabismus	Hemifacial spasm
Crow's feet	Blepharospasm	Facial asymmetry
Forehead lines	Nystagmus	Oromandibular dystonia
Perioral lines		Cervical dystonia
Platysmal bands		Spasmodic torticollis
Brow ptosis		Achalasia
Palmar hyperhidrosis		Gustatory sweating
Axillary hyperhidrosis		Synkinesia
		Hyperlacrimation

muscle activity such as cervical dystonia,[12] spasmodic torticollis,[13,14] and achalasia[15] (Table I).

BTX use in the field of dermatology (Table I) began in the early 1990s when improvement in facial wrinkling was observed. While treating hemifacial spasm patients with BTX-A, Borodic, Cheney, and McKenna[16] reported a concomitant unilateral decrease in facial wrinkling. Carruthers and Carruthers[17,18] treated patients with blepharospasm and noticed an improvement in glabellar frown lines. These initial findings and subsequent clinical studies popularized BTX-A as a safe and effective treatment for hyperfunctional glabellar frown lines, crow's feet, and forehead lines.[17-23] More recently, BTX-A has been used to treat platysmal bands,[24,25] brow position,[19,26] and palmar[27] and axillary hyperhidrosis.[28]

NEUROMUSCULAR TRANSMISSION

Cholinergic neurotransmission involves 6 steps: synthesis, storage, release, binding, degradation, and recycling of acetylcholine[29,30] (Fig 1). Choline is first transported from the extracellular fluid into the cholinergic neuron's cytoplasm by a carrier system that cotransports sodium. Choline reacts enzymatically with acetylcholine CoA to form acetylcholine, which is then transported into synaptic vesicles where it is stored in granules. When an action potential arrives at a nerve ending, voltage-sensitive calcium channels in the presynaptic membrane open causing an increase in the concentration of intracellular calcium. Elevated calcium levels promote the docking and fusion of synaptic vesicles with the cell membrane via a complex mechanism involving protein isoforms, culminating in the release of acetylcholine. Acetylcholine then diffuses across the synaptic space and binds to postsynaptic nicotinic receptors on the muscle fiber. This binding activates a second messenger system that results in muscle contraction. Acetylcholine is rapidly cleaved into choline and acetate by acetylcholinesterase. Choline may be recycled by a high-affinity transport system that pulls the molecule back into the neuron.

TOXIN STRUCTURE

Botulinum neurotoxins are produced as inactive polypeptides of 150 kd, which are cleaved by trypsin-like bacterial protease to generate the di-chain active form of the toxin. The proportion of single to di-chain toxin is dependent on the toxin's serotype and whether or not the bacterial strain expresses the appropriate protease.[31] The 100-kd heavy (H) chains and the 50-kd light (L) chains are linked together by heat-labile disulfide bonds and noncovalent forces.[32] The H and L chains dissociate with heat and boiling, which inactivates the toxin because neurotoxicity requires both H and L chains.[33]

MOLECULAR ACTIONS

All serotypes act on the peripheral nervous system where they inhibit release of acetylcholine from the presynaptic terminal of the neuromuscular junction.[34] Toxins may bind to nerve terminals at autonomic cholinergic ganglia with autonomic effects, but only in very large doses. It is unlikely that therapeutic doses are associated with any significant autonomic adverse reactions.[33] There are three steps involved in neurotoxicity[35] (Fig 1).

Binding

The first step is the irreversible binding of BTX to presynaptic cholinergic receptors via the H chain's 50-kd carboxy-terminal.[36-38] The associated binding sites have not been clearly characterized. Previous studies have suggested that distinct receptors exist for different BTX serotypes.[36] This view has been challenged by the isolation of a highly conserved synaptic vesicle protein, synaptotagmin, which binds to BTX-A, BTX-B, and BTX-E.[39]

Internalization

The second step involves internalization of the neurotoxin through a receptor-mediated endocytosis.[40] This process is independent of calcium and partially dependent on nerve stimulation.[41,42] After

Table II. Target substrates of botulinum toxin

Toxin type	Substrate	Reference No(s).
BTX-A	SNAP-25	122
BTX-B	VAMP/synaptobrevin	123
BTX-C	SNAP-25 and Syntaxin	124, 125
BTX-D	VAMP/synaptobrevin	122
BTX-E	SNAP-25	122
BTX-F	VAMP/synaptobrevin	126
BTX-G	VAMP-synaptobrevin	127

internalization, the disulfide bond is cleaved by an unknown mechanism. The H chain's 50-kd amino-terminal is associated with ionic channel formation and translocation of the L-chain from the endosome into the neuronal cytoplasm.[43]

Neuromuscular blockade

The third step is neuromuscular blockade. Within the synapse, protein isoforms form a complex platform necessary for the docking, fusion, and release of acetylcholine vesicles through the cell membrane.[44,45] These protein isoforms are vesicle associated membrane protein (VAMP, also known as synaptobrevin), synaptosomal associated protein (SNAP-25), and syntaxin. The L- chain of each toxin, which contains a highly specific zinc-endopeptidase with proteolytic activity concentrated at its aminoterminal, cleaves a single protein isoform at a single site[33] (Table II). The only exception is BTX-C, which cleaves two proteins.

DRUG AND DISEASE INTERACTION

Several drugs act on the neuromuscular junction and interfere with the effect of BTX (Table III). BTX may be potentiated by aminoglycoside antibiotics.[46] Large doses of aminoglycosides such as kanamycin, streptomycin, and gentamicin can prevent the release of acetylcholine from nerve endings and produce a botulism-like clinical syndrome.[47] This effect may be related to calcium channel blockade.[48,49] Symptoms rapidly abate as the offending drug is eliminated from the body.

Aminoquinolines (chloroquine and hydroxychloroquine) antagonize the onset of paralysis from BTX by acting either at the cell membrane to inhibit toxin binding or internalization, or in the cell interior to inhibit lysosomal processing of toxin.[50] Cyclosporine has been reported to cause neuromuscular blockade characterized by muscle weakness and ventilatory failure.[51] The precise mechanism of action is unknown but may be the result of anti-inflammatory or immunosuppressive effects on the muscle or presynaptic calcium channel blockade.[52]

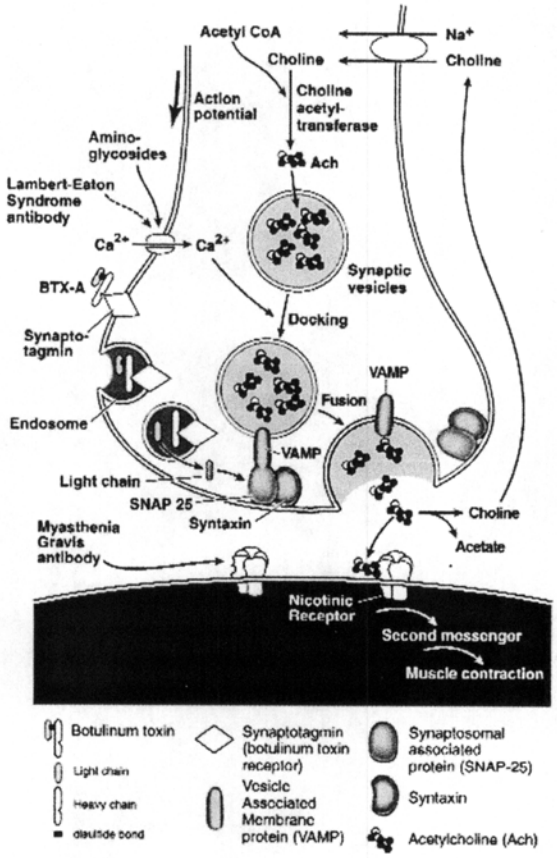

Fig 1. Cholinergic neurotransmission and botulinum toxin's mechanism of action.

D-Penicillamine can trigger the formation of acetylcholine receptor antibodies in immunologically predisposed individuals.[53] A small percentage of patients with rheumatoid arthritis who receive D-penicillamine develop acetylcholine receptor antibodies[54] and symptoms of myasthenia gravis.[55] Both the symptoms and antibodies remit within a few months after drug cessation.[56] The antibody repertoire in the sera of patients with myasthenia gravis and D-penicillamine–induced myasthenia gravis resembles each other.[57]

A few nondermatologic drugs block cholinergic transmission between motor nerve endings and the postsynaptic nicotinic receptors on the neuromuscular end-plate. They act either as competitive, antagonist blockers (tubocurarine, pancuronium, and gallamine) or as agonist blockers (succinylcholine). The antagonist blockers compete with acetylcholine for receptor binding sites. A sufficient amount of drug will block the acetylcholine receptors, thereby preventing muscle depolarization. The mechanism of agonist blockers resembles the

Table III. Drug and disease interactions

Drug/disease	Mechanisms
Presynaptic nerve terminal	
Aminoglycosides (kanamycin, streptomycin, gentamicin)	Calcium channel blockade
Aminoquinolines (chloroquine, hydroxychloroquine)	Inhibit botulinum toxin binding to synaptotagmin or lysosomal processing of toxin
Cyclosporine	Calcium channel blockade
Lambert-Eaton syndrome	Cross-reacting, tumor antigen antibodies against calcium channels
Postsynaptic nerve terminal	
Myasthenia gravis	Autoantibodies to the nicotinic acetylcholine receptor
D-Penicillamine	Triggers the formation of nicotinic acetylcholine receptor antibodies
Tubocurarine, pancuronium, gallamine	Postsynaptic acetylcholine antagonist blockers
Succinylcholine	Postsynaptic acetylcholine agonist blocker

depolarizing action of acetylcholine. However, the muscle repolarization from succinylcholine is much slower than acetycholine, causing a delay in muscle contraction.[58]

BTX use is contraindicated in patients with disorders of neuromuscular transmission (eg, Lambert-Eaton syndrome and myasthenia gravis). In Lambert-Eaton syndrome, antibodies directed against tumor antigens cross-react with voltage-gated calcium channels involved in acetylcholine release, leading to a disturbance of neuromuscular transmission.[59] Weakness in myasthenia gravis is caused by an antibody-induced internalization and degradation of acetylcholine receptors.[60,61]

ASSAYS AND PHARMACOLOGIC ACTIONS

Muscle assays

Several assays are invaluable in elucidating the pharmacologic action of BTX. The mouse phrenic nerve diaphragm model established the multi-step hypothesis of BTX.[62,63] Toxin radiolabeling coupled with histologic analysis delineated the relationship among toxins, receptor sites, and motor end plates. It demonstrated that nerves of actively contracting muscles bind BTX more rapidly[41] at an average of 32 to 64 minutes.[48] This phenomenon supports the directive for patients to exercise their injected muscles for up to an hour after BTX injection.

The mouse hypoglossal nerve assays showed that unlike doxorubicin, which causes toxicity to the neuron cell body by retrograde transport,[64,65] BTX does not induce motorneuron death.[66] Rather, it causes a chemical denervation to the neuromuscular junction.[67]

Studies of muscle fibers from human blepharospasm patients demonstrated that denervation is reversible. Using acetylcholinesterase stain to assess denervation of striated muscle after injection of BTX, Borodic et al[68] observed that denervation is accompanied by spreading of the acetylcholinesterase activity to cover most of the exposed sarcolemma. After 4 to 5 months, the distribution of acetylcholinesterase activity reverts to its normal pattern. Recovery from denervation occurs by neurogenesis, with the formation of axonal sprouts within 10 days and new motor end plates.[69] A reconnection between nerve terminals and muscle motor end plates is therefore established.

The rabbit longissimus dorsi muscle assay showed that muscle atrophy is also reversible. Localized muscle atrophy is seen within 2 weeks of toxin injection, continues for about 4 weeks, and then reverses.[70] Similar reversible denervation atrophy has been seen in the orbicularis oculi muscles of blepharospasm patients treated with BTX-A.[68,71] Clinical recovery of function requires about 3 to 6 months, at which time the muscle returns to about 70% to 80% of its original bulk.[33] Sprouting and remodeling may continue for up to 3 years.[72,73]

Bioassay

The mouse biologic assay is currently the only accepted quantitative method for the detection of *Clostridium* toxins in culture, serum, and food samples,[74] and antitoxin standardization.[75] It is the most sensitive and specific measurement of BTX activity. Factors affecting the assay include sex, strain, species, and the route of administration (intraperitoneal vs intramuscular).[76]

One mouse unit (MU) is defined as the median intraperitoneal dose required to kill 50% of a batch of 18 to 20 g of female Swiss-Webster mice (LD_{50}) over 3 to 4 days.[77-79] The original assay measured the LD_{50} in monkeys, which was found to be less narrow than the lethal dose effects in mice.[7] The LD_{50} of

BTX-A for humans, extrapolated from experiments in monkeys, has been estimated to be about 40 MU/kg.[80] For a 70-kg man, the LD_{50} falls in the range of 2500 to 3000 MU. Species variability in their sensitivity to the toxin prevents an accurate calculation of the toxic dose for humans.[33] Nevertheless, there is a wide safety margin in therapeutic use. For example, the average number of units required for the treatment of glabellar frown lines is about 30 MU.

The greatest pitfall of the mouse bioassay is that it does not provide an accurate characterization of the potency of BTX in humans.[81] Clinical potency is dependent upon the toxin's dose, volume, and targeted muscle.

Immunoassays

Because of the expenses for animals and testing facilities and issues of ethical consideration, in vitro immunoassays (immunodiffusion assay, hemagglutination assay, radioimmunoassay, enzyme-linked immunosorbent assay [ELISA]) have also been developed for rapid detection and quantitation of BTX. Immunodiffusion and hemagglutination assays, which use a single monoclonal antibody, are still not sensitive enough to replace the mouse bioassay.[82] The requirements to radiolabel toxin and to have suitable radiologic facilities make radioimmunoassay inapplicable for the routine testing of samples.

ELISA has the greatest potential as a replacement for the bioassay. The standard ELISA technique using polyclonal antibodies is highly specific, reasonably rapid, and can be applied to the testing of a large number of specimens.[74] The sensitivity of ELISA can be further improved by a redox cycle amplification system[83] or an enzyme-linked coagulation amplification system.[84] The major disadvantage of all ELISA-based assays is the inability to differentiate between active and inactive toxin.[74] A specific assay based on the BTX endopeptidase activity is being investigated. Unlike ELISA-based assays, this new assay directly measures the biologic activity of the toxin.[85,86]

These in vitro assays may not totally replace the mouse bioassay because they do not give a measurement of other parameters such as cell binding and internalization, which contribute to overall toxicity and therapeutic potency. Therefore the mouse bioassay is currently the gold standard for toxin detection and standardization.

DETERMINANTS OF CLINICAL RESPONSE

There is no clinical standardization on the use of BTX-A. Factors that affect clinical response are commercial preparations, anatomy, dose and response relationship, storage, and immunogenicity.

Preparations

There are two commercially available BTX-A preparations: Botox (Allergan, Inc, Irvine, Calif) and Dysport (Speywood Pharmaceuticals, Maidenhead, England). Only Botox is currently available in the United States. The Botox unit is 3 to 5 times as potent as the Dysport unit.[14]

The currently available batch of Botox, derived from toxins prepared by Allergan, Inc, in 1997, has replaced the old batch originally prepared by Schantz in 1979.[87] Botox is a sterile, lyophilized (vacuum-dried) form of purified BTX type A, produced from a culture of the Hall strain of *C botulinum* grown in a medium containing N-Z amine and yeast extract. It is isolated from the culture solution by a series of acid precipitations to a crystalline complex consisting of the active high molecular weight toxin protein and an associated hemagglutinin protein. The crystalline complex is then redissolved in a solution containing saline and albumin for stability, and sterile-filtered before vacuum-drying. In the Schantz preparation, 1 MU of the crystalline protein complex weighed about 0.043 ng.[78] The amount of chromatographically purified botulinum A toxin was approximately 0.006 ng.[88] The new batch has only 20% of the protein content of the old batch.

Each vial of Botox contains 100 MU of *C botulinum* toxin type A (with 10% variability), 0.5 mg of human albumin, and 0.9 mg of sodium chloride in a sterile, vacuum-dried form without a preservative. The average cost per vial is about $370. The vials are stored in the freezer before reconstitution for clinical use. The recommended diluent is nonpreserved normal saline. The use of preserved saline during reconstitution may alter the dose response.[17] Excessive shaking and bubbling during reconstitution may inactivate the toxin. After reconstitution, the product should be stored in the refrigerator at 2°C to 8°C.[89]

Dysport (BTX type A) preparation is different in terms of MU, chemical properties, biologic activities, and weight.[90] It is supplied in 500 MU vials, produced by column-based purification rather than by the precipitation technique used for Botox, and may be stored at room temperature. The recommended diluent is also unpreserved normal saline.[91] It has been used successfully for blepharospasm,[90] torticollis,[92] hemifacial spasm,[93] and hyperfunctional facial lines.[91]

Anatomy

Treatment should be tailored to the individual patient. The size and orientation of muscle fibers vary between different anatomic regions and sexes. Hyperfunctional lines are perpendicular to the sum vectors of muscle forces. All the superficial muscles

of the face are part of the superficial musculoaponeurotic system.[94]

The muscles in the glabellar region are intertwined. The medial fibers of the frontalis muscles are the only medial brow elevators. The medial brow depressors consist of the corrugator supercilii, depressor supercilii, orbital portion of the orbicularis oculi, and procerus. The strong corrugator muscles contribute most to the frown lines and require higher doses of BTX for muscle paralysis.[20] Paralysis of the medial brow depressor muscles reduces the vertical glabellar wrinkles and horizontal nasal root wrinkles with a concomitant medial eyebrow lift (Huang et al, manuscript accepted for publication).

The frontalis muscle raises the medial and lateral brows and contributes to horizontal forehead line wrinkles. Because the frontalis spans the entire forehead, multiple point injections are required for effective paralysis.[95] The medial fibers, being more fibrous than the lateral fibers, require larger doses.[94] Injection of lower frontalis fibers too close to the lateral brow may lead to brow ptosis.[21]

The function of the orbicularis oculi muscle is to close the eye. Contraction of the lateral orbicularis oculi muscle gives rise to crow's feet. Because of the superficial position of the orbicularis oculi muscle, injection in this region is usually directed into the dermal or subcutaneous plane along the outer orbital rim peripheral to the lateral canthus. This technique also helps to minimize toxin diffusion and paralysis of neighboring muscles. For example, unintentionally paralyzing the levator labii superioris muscle would result in temporary eyelid ptosis. Innervation studies of the orbicularis oculi muscle have demonstrated a diffuse distribution of neuromuscular junctions.[96] Therefore multiple injection points are more likely to provide a satisfying outcome than a total equivalent dose given into one injection point.

Women's brows are frequently arched above the orbital rim. Men tend to have a more horizontal brow. In patients with horizontally oriented brows and deep frown lines, an injection 1 cm above the orbital rim in the midpupillary line may be needed to paralyze the tail of the corrugator and the temporal orbicularis.[97]

The depths of all dynamic lines may be augmented by photodamage. As a result, a complete correction of the lines may not be possible. However, with relaxation of the muscle and protection from photodamage, dermal remodeling may change with time. For a review of other anatomic Botox sites (lips, platysma, axilla, and palms and soles), the reader should refer to more in-depth articles.[23,25,27,28,98]

Dose and response relationships

The effect of Botox is dependent on the location, concentration, and volume of solution that is injected. The art of using Botox comes from choosing the desired weakening of muscle contraction without causing unwanted muscle paralysis. Reported concentrations of solutions used for cosmetic indications range from 1 MU/0.1 to 10 MU/0.1 mL. Reported volumes injected in each location range from 0.025 to 1.0 mL per site. Typically volume and concentration are increased to correlate with the size of the treated muscle. Dosing in general is still rather arbitrary and based on the experience of the physician.[99]

In attempt to examine the relationship between dose, volume, and targeted muscles, Borodic et al[100] used rabbit longissimus dorsi and acetylcholinesterase staining as indices of denervation and showed that the size of the denervation field is determined by dose and volume. Specifically, a single injection of 10 MU per 0.1 mL causes a toxin spread of 4.5 cm. Using rat anterior tibialis muscle and periodic acid–Schiff staining to measure glycogen depletion and quantify paralysis, Shaari and Sanders[101] found that dose was a stronger predictor of area of paralysis than volume and the effect of BTX was greatest when injected closest to the motor end plate. Injecting into the crow's feet area with a total dose of 6 to 15 MU with 3 injection sites, Carruthers[102] noted the effect of the toxin spread to be at least 1 cm. Using the glabellar frown lines as a model to evaluate the dose-response, Hankins, Strimling, and Rogers[103] concluded that the threshold for a demonstrable response to BTX is between a total dose of 5 and 12.5 MU or 1 to 2.5 MU per injection site (a total of 5 injection sites), whereas an effective starting dose is between a total dose of 12.5 and 20 MU or 2.5 to 4 MU per injection site, with a duration of 2 to 5 months. There was no statistically significant difference in safety or efficacy for concentrations ranging from 50 to 200 MU/mL of BTX.

In summary, to achieve maximal dose response and minimize side effects, the clinician should use the most effective dose at the smallest volume. At a particular injection site, small volume and high dose are superior to large volume and low dose. Small volume and high dose tend to localize the toxin and contain the biologic effect of muscle paralysis.[95] Large volume and low dose weaken the muscle and may produce an overall smoothing effect[20] with a concomitant risk of toxin spread to adjacent muscles.[22]

Storage

Storage after reconstitution may affect the biologic potency of Botox. The current Food and Drug

Administration–approved product labeling recommends that it should be used within 4 hours of reconstitution with normal saline,[89] which makes Botox use somewhat cumbersome in clinical settings.

Several studies have investigated the relationship between storage and potency. One study using the mouse bioassay showed no loss of activity 6 hours after reconstitution at room temperature. However, when left for 12 hours, a loss of up to 44% activity was observed. Refreezing the toxin after reconstitution was reported to cause approximately 70% loss of bioactivity after 1 to 2 weeks.[104] In a human extensor digitorum brevis model, Sloop, Cole, and Escutin[105] showed no loss of potency in the reconstituted toxin after refrigeration or refreezing for 2 weeks. Using time stored diluted Botox, Lowe[91] observed a 50% decrease in the efficacy of Botox to reduce hyperfunctional facial lines after 1 week. Another study demonstrated that toxin at 10 MU/mL reconstituted 30 days before injection produced paralysis of facial muscle tone equivalent to that of freshly mixed toxin.[20] All studies used unpreserved normal saline for reconstitution. Although there are no standardized guidelines for storage, most clinicians do not refrigerate the toxin for more than 1 week. Refrigeration for less than 24 hours is optimal, and refreezing is discouraged.[106]

Immunogenicity

The toxin's immunologic properties can lead to the stimulation of antibody production, potentially rendering further treatments ineffective. The minimum dose and injection schedule required to induce antibody formation are unknown. Immunogenicity has been shown to be dependent on dose per injection session, cumulative dose, and frequency of administration.

A study by Biglan et al[107] revealed absence of antibody formation in patients who received less than 50 MU per injection session. Gonnering[108] reported antibody response in patients with facial spasm syndromes receiving doses in the 150 to 300 MU range but not in those receiving doses of up to 52.5 MU per session over a period of 163 weeks. Testing antibody production in cervical and oromandibular patients, Jankovic and Schwartz[109] observed a statistically significant difference between patients with antibodies and a mean cumulative dose of 1709 MU and patients without antibodies and a mean cumulative dose of 1066 MU. Zuber et al[110] noted a 3% prevalence rate in focal dystonia patients receiving a greater than 50 ng (~1162 MU) cumulative dose. Study of torticollis patients treated with doses ranging from 150 to 300 MU demonstrated a 4.3% prevalence of neutralizing antibody, and also noted that BTX-resistant patients received more frequent injections and more booster injections 2 to 3 weeks after treatment.[111] To date, antibody formation has not been reported in patients treated for blepharospasm or dermatologic uses.[18]

The most widely used test for antibody detection is the mouse neutralization assay (Northview Pacific Labs, Berkeley, Calif).[112] Antibodies from human serum and BTX-A are coadministered to mice. The binding of antibodies to toxin protects mice from the toxin's lethal effects, and this neutralization is quantified. The mouse test is time-consuming and expensive.[33] More rapid immunoassays have also been used to detect the anti-BTX antibody. Their main drawbacks are lower specificity[113] and lack of correlation between detected antibodies and clinical resistance.[84] A combination of the mouse neutralization assay and an immunoassay would probably provide the most sensitive and specific test.

To minimize antibody resistance, one should use the smallest possible effective dose, use treatment intervals of at least 3 months, and avoid booster injections.[111] Moreover, shorter intervals between injections may not produce as prolonged a functional effect as longer intervals.[114] Patients with BTX-A resistance may benefit from injections with BTX-B (Athena Neurosciences, San Francisco),[12] BTX-C (Speywood Pharmaceuticals),[115] or BTX-F (Speywood Pharmaceuticals).[116] Because these other serotypes have different potencies, their duration of effect would vary.[40] BTX-B, BTX-C, and BTX-F are currently under clinical investigation; the only commercially available toxin for routine clinical use at present is BTX-A.

PRECAUTIONS

Botox is a Pregnancy Category C drug, which means that animal reproduction studies have not been conducted and that the human teratogenic effects of Botox are unknown. To date, there have been no reports of teratogenicity.[117] It has not been determined whether this drug is excreted in human milk. Use of BTX in pregnant and lactating women is contraindicated. Caution should be exercised when using BTX in children younger than 12 years old.[89]

ADVERSE EFFECTS

After a decade of therapeutic application of the toxin, no anaphylaxis or deaths attributable to Botox have been reported.[40] Uncommon adverse effects are ptosis, ectropion, diplopia, eyelid drooping, hematoma, and bruising.

Postinjection ptosis results from toxin diffusion through the orbital septum paralyzing the levator palpebrae superioris muscle.[117,118] Transient ptosis is extremely rare and is usually minimal (1-2 mm of

ptosis) and short-lived (lasting approximately 2 weeks).[117] Techniques to help avoid this complication include using low injection volume, accurately placing the needle 1 cm above the central eyebrow, aiming the needle upward and horizontally, injecting slowly to limit toxin spread, and exercising frequent muscle contraction for 30 minutes after the injection.[21] Ptosis can be treated with apraclonidine 5% (Lopidine, Alcon, Inc, Dallas, Tex) but this should be done with caution. This α-adrenergic glaucoma medication causes contraction of the Muller's muscle, which is situated beneath the levator muscle of the upper eyelid, and results in elevation of the lash margin.[97] Ectropion, diplopia, and drooping of the lateral lower eyelid can be avoided by injecting at least 1 cm peripheral to the bony orbit.[97] Local hematoma and bruising are prevented by immediate digital pressure on the injection site. Other minor side effects are pain and temporary headaches.[119]

Long-term effects of Botox may include local changes in muscle fiber size and electromyographic abnormalities. These changes do not appear to have any clinical significance.[40] There are no remote clinical effects of Botox, though local injection can produce subclinical electromyographic changes in uninjected distant muscles. The mechanism of this action is unclear.[120]

BTX does not cross the blood-brain barrier and therefore has no central nervous system effects.[121] Whether intact BTX reaches the central nervous system after intramuscular injection by retrograde transport is undetermined and unlikely to be clinically important.[40]

CONCLUSION

This article reviews the pharmacology of BTX and its past and present uses. Botox has been shown to be a safe and effective treatment. Standardization of administrative techniques with emphasis on maximizing efficacy and minimizing complications will be useful in achieving consistent beneficial results in the future. As the use of BTX evolves, the field of dermatology will build upon this foundation of knowledge and explore more creative applications.

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